FERMENTS FOR CARCINOMATOUS PROTEIN IN THE BLOOD IN CARCINOMA.*

GEORGE F. DICK.

(From the Memorial Institute for Infectious Diseases, Chicago.)

By means of the method of dialysis devised by Abderhalden, Brockmann,[1] Frank and Heimann,[2] and Epstein[3] have found that the serum of the blood in cases of carcinoma contains ferments which act on the proteins of carcinoma tissue. Such ferments were not found in the serum of persons free from carcinoma.

I have made an attempt to estimate quantitatively the amount of proteolysis in carcinomatous material when acted on by carcinomatous and normal serum under comparable conditions. At first the optical method was used. In this case the carcinoma tissue ("antigen") was prepared by grinding with sand and extracting with salt solution over night in the icebox. The extract was centrifugated and the fluid passed through Berkefeld filters and heated to 60° C. for 10–15 minutes in order to destroy the ferments in the material itself. Of such extracts 10 c.c. were mixed with 2 c.c. of serum and the optical rotation determined at once and after incubation for 3 hrs. at 37° C. The results follow:

	1. Carcinoma of Uterus.		2. Carcinoma of Uterus.		3. Carcinoma of Breast.	
	Patient's Serum	Normal Serum	Patient's Serum	Normal Serum	Patient's Serum	Norma Serum
At once.....	50′	53′	35′	40′	43′	40′
After incubation	45′	54′	31′	39′	39′	39′
	5′	1′	4′	1′	4′	1′

In these three tests there is evidence of some proteolysis by the carcinomatous serum and of less by the normal serum, altho the differences in the normal readings are not beyond the limits of unavoidable error.

The preparations of the material for the optical method being difficult, other tests were made by the method of titration of amino

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[1] *Lancet*, 1913, 185, p. 1384.

[2] *Berl. klin. Wchnschr.*, 1913, 50, p. 631.

[3] *Wien. klin. Wchnschr.*, 1913, 26, p. 650.

acids (Henriques, Sorensen). The carcinomatous tissue was prepared as before but the extracts were not filtered. For each complete test about 5 gm. of tumor were used made up into 40 c.c. of extract, 20 c.c. of which were mixed with 4 c.c. of carcinomatous serum and 20 c.c. with 4 c.c. of normal serum. To each mixture were added 3 c.c. of toluene, with thorough shaking to prevent bacterial growth. The amounts of amino acids in 10 c.c. of each mixture were now determined in terms of $\frac{2}{10}$ sodium hydrate before and after incubating for 24 hrs., the difference in the amount of amino acids in the 2 parts of the mixtures representing the amount of proteolysis taking place during the incubation. The 10 c.c. tested before incubation were removed by means of a pipette as soon as the toluene formed a layer at the top. In each test the 10 c.c. of serum mixture were measured into a 200 c.c. Erlenmeyer flask; to this, 100 c.c. of distilled water and 5 drops of 0.5 per cent solution of phenolphthalein were added and then N/10 NaOH until a faint pink tinge appeared. Next 5 c.c. of formalin were diluted to 25 c.c. with distilled water and 5 drops of 0.5 per cent solution of phenolphthalein added. The solution was brought to a faint pink color by adding N/10 NaOH. The neutral formalin was then added to the neutral carcinoma-serum mixture and the resulting acidity measured by a burette reading of the amount of N/10 NaOH necessary to restore the pink color. The mixtures of carcinoma extract and serum were made at icebox temperature so as to prevent as much action as possible before the first amino acid titration.

The results of the experiments are given in terms of N/10 NaOH.

	4. Carcinoma of Uterus.		5. Carcinoma of Uterus.		6. Carcinoma of Uterus with Omental Metastasis; Extract from Metastasis.	
	Patient's Serum	Normal Serum	Patient's Serum	Normal Serum	Patient's Serum	
1st titration....	2 c.c.	2.1 c.c.	3.40 c.c.	3.4 c.c.	1.05 c.c.	No Control
2d "	2.5 "	2.2 "	3.95 "	3.9 "	0.95 "	
	0.5 "	0.1 "	0.55 "	0.5 "	0.1 "	

	7. Recurrent Carcinoma of Uterus.		8. Carcinoma of Uterus.		9. Carcinoma of Uterus.		
	Patient's Serum	Normal Serum	Patient's Serum	Normal Serum	Patient's Serum	Serum of Case 10.—Carcinoma of Uterus	Normal Serum
1st titration..	2.25 c.c.	2.25 c.c.	4.95 c.c.	4.75 c.c.	2.20 c.c.	2.4 c.c.	2.4 c.c.
2d " ..	2.20 "	2.30 "	4.30 "	5.15 "	2.95 "	2.9 "	2.9 "
	0.05 "	0.05 "	0.65 "	0.60 "	0.75 "	0.5 "	0.5 "

Inasmuch as there was a well-marked change from the normal in Case 9 a comparison was made between that serum and the serum of Case 10. Instead of extract of uterine carcinoma an extract from a carcinoma of the breast (Case 3) was used to see if there would be the same difference in this case also. The action of all 3 sera was tried also on a 2 per cent gelatin peptone solution at the same time to find out whether or not a difference existed between the action of carcinoma serum on carcinoma and other protein material. The results follow:

Extract of Carcinoma of Breast.			Gelatin-Peptone Solution.		
Serum of Case 9.— Carcinoma of Uterus.	Serum of Case 10.— Carcinoma of Uterus.	Normal Serum	Serum 9	Serum 10	Normal Serum
2.95 c.c.	3.1 c.c.	2.95 c.c.	0.95 c.c.	1.00 c.c.	1.00 c.c.
3.45 "	5.0 "	3.25 "	1.00 "	1.05 "	1.05 "
0.55 "	1.9 "	0.30 "	0.05 "	0.05 "	0.05 "

From these figures it will be seen that in Case 9 there was a difference from the normal of 0.55 c.c. which would indicate a slightly lower proteolytic on breast carcinoma than on the homologous carcinoma, but in Case 10 there was a difference from the normal of 1.9 c.c., indicating that more proteolysis occurred in the breast carcinoma than in the uterine carcinoma. Experiments with gelatin, on the other hand, showed an entire lack of proteolysis by all 3 sera.

It has been shown[1] that proteolytic processes occur during hemolysis by immune serum and that a certain parallelism exists between the proteolytic power of the blood in pneumonia and the complement content of the blood. Hence, simultaneously with the comparison of the proteolytic power of the blood in the experiments described, a comparison of the complement content of the sera was made. In some cases this was impossible on account of the presence of an amboceptor for the corpuscles used (sheep). The comparisons in the complement content of the blood were made by adding graded amounts of normal and carcinoma sera to constant quantities (0.2 c.c.) of a 5 per cent suspension of sheep corpuscles with an excess of amboceptor equal to 10 times the minimum quantity necessary to hemolyze 0.2 c.c. of corpuscles in the presence of 0.01 c.c. of normal human serum.

[1] Dick, *Jour. Infect. Dis.*, 1911, 9, p. 282, and 1912, 10, p. 383.

The complement of the different specimens of serum is given below in terms of the number of times the strength of normal serum taken as one. Case 1 was 5 times the normal in complement content; Case 2, 1; Case 3, $1\frac{2}{3}$; Case 4, $1\frac{2}{3}$; Case 8, $1\frac{2}{3}$; Case 10, 3. In 3 cases in which the proteolytic power was not estimated the complement was $2\frac{1}{2}$, 1, and $1\frac{2}{3}$ times that of normal serum. It will be seen that as a rule the complement content of the blood in carcinoma is higher than that of normal serum.

SUMMARY.

By the titration method of estimating amino acids, ferments capable of splitting protein from carcinomatous tissue are demonstrable in the blood serum of patients with carcinoma.

The power of the blood serum of carcinomatous patients to split carcinomatous protein does not differ from the normal with the same constancy and to the same degree when estimated by amino acid titration as indicated by the results obtained with the dialysis method of Abderhalden.

Serum capable of splitting protein from carcinoma of the uterus was also capable of splitting protein from carcinoma of the breast, but not mixtures of gelatin and Witte's peptone.

The complement content of the blood serum of a carcinoma patient is as a rule higher than normal.